Evaluation of continuous aspiration of subglottic secretion in an *in vivo* study*

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Objective: Continuous aspiration of subglottic secretions (CASS) is believed to lower the incidence of ventilator-associated pneumonia. Animal studies to establish safety and efficacy of CASS have not been conducted.

Design: Prospective randomized animal study.

Setting: Animal-research facility at the U.S. National Institutes of Health.

Subjects: Twenty-two sheep.

Interventions: Sheep were randomized into three groups. In group C (control), eight sheep were kept prone, intubated with a standard endotracheal tube (ETT), and mechanically ventilated for 72 hrs with head and ETT elevated at an angle of 30°. In group CASS-HU (CASS, head up), seven sheep were managed as group C and intubated with a Hi-Lo Evac, Mallinckrodt ETT (CASS suction kept at ≤20 mm Hg). In group CASS-HD (CASS, head down), seven sheep were kept prone with CASS, and the ETT and trachea were horizontal to promote spontaneous drainage of mucus from the ETT.

Measurements and results: The lower respiratory tract in the CASS-HU group was heavily colonized in all seven sheep (median 4.6×10^9, range, 1.5×10^8 to 7.9×10^9 colony-forming units/g), with a reduction of lung bacterial colonization compared with the C group ($p = .05$). In group CASS-HD, the lower respiratory tract was not colonized in six of seven sheep. One sheep showed low levels of bacterial growth (median, 0; range, $0–2.2 \times 10^5$). At autopsy, in all 14 sheep with CASS, we found tracheal mucosal injury of different degrees of severity at the level of the suction port of the ETT.

Conclusions: In group CASS-HU, regardless of finding a marginal decrease of the bacterial colonization of the lower airways, there was pervasive trachea-bronchial-lung bacterial colonization. Second, there was minimal, or absent, bacterial colonization when the orientation of the CASS ETT was at, or just below, horizontal. Third, there was widespread injury to tracheal mucosa/submucosa from the use of CASS.

Note that results of studies conducted in an animal model are always difficult to extrapolate to the clinical practice due to anatomical and functional differences. (Crit Care Med 2004; 32:2071–2078)

KEY WORDS: mechanical ventilation; aspiration; ventilator-associated pneumonia; endotracheal tube; continuous aspiration of subglottic secretions

Ventilator-associated pneumonia (VAP) carries a high morbidity and mortality rate in patients intubated and mechanically ventilated. Preventive strategies to reduce bacterial colonization of the aerodigestive tract and aspiration of contaminated secretions are believed to reduce the incidence of VAP (1–4).

Over the past 10 yrs, four randomized clinical trials evaluated the role of aspiration of subglottic secretions: The impact on pneumonia was mixed, and mortality rate did not change (5–8). Surprisingly, no previous laboratory animal studies had been conducted before those clinical trials assessing safety and efficacy of this new clinical device (Hi-Lo Evac, Mallinckrodt, St. Louis, MO). Nevertheless, continuous aspiration of subglottic secretions (CASS) is now listed in many guidelines for the prevention of VAP (4, 9, 10), without apparently changing the clinical practice of many intensive care units (11, 12).

Semirecumbency is another nonpharmacologic strategy to prevent VAP, broadly used in intensive care units. Drakulovic et al. (13) showed that semirecumbent body position (45°) reduces the frequency and risk of nosocomial pneumonia (14). However, recent clinical trials did not confirm those results (15, 16).

Recently, we have shown that the horizontal orientation of the endotracheal tube (ETT), and of the trachea, prevented accumulation of secretions above the ETT cuff, prevented leakage of oropharyngeal contents around the ETT cuff, and promoted the outward drainage of bacteria-laden mucus from within the ETT (17, 18). In the same animal model, with sheep mechanically ventilated for 72 hrs, we explored safety and efficacy of CASS in preventing bacterial colonization of the lower respiratory tract with the ETT both horizontal and elevated 30° above horizontal, as in patients lying supine or semirecumbent.

MATERIALS AND METHODS

End Points

The primary end point of this laboratory investigation was whether the use of CASS

*See also p. 2160.

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Supported, in part, by the National Heart, Lung and Blood Institute, Division of Intramural Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD.

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DOI: 10.1097/01.CCM.0000142575.86468.9B

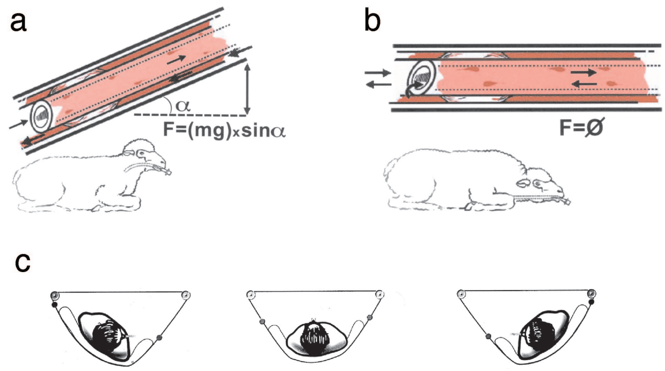

Figure 1. *a*, in the control and CASS HU (continuous aspiration of subglottic secretions head up) groups, head and the endotracheal tube (ETT) were elevated 30° above horizontal. Note that in this position in sheep (as in the human), some fluid and bacteria-laden secretions from within the ETT, and from possible leakage around the inflated cuff of the ETT, can drain into the trachea and the lungs, irrespective of best efforts to remove the bulk of such secretions/aspirate through suctioning. *b*, in the CASS HD (continuous aspiration of subglottic secretions head down) group, sheep were prone with the ETT and the trachea horizontal, or just below horizontal. Note that the ETT with the orientation horizontal, or below horizontal, the gravitational force to assist outward drainage of heavily colonized mucous/secretions from within the ETT is facilitated. Normal tracheal mucociliary clearance ensures that whatever oropharyngeal/gastric contents may have entered the trachea will also be expelled into the ETT, then drained outward, and removed by suctioning. *c*, to keep the ETT and trachea horizontal, we fabricated a prototype rotating bed; here shown as an artist drawing. With this system, the patient can be moved intermittently from the left lateral position to the right lateral position. On the left and on the right of the figure, the body of the patient lies in semilateral position of approximately 45°, with the head rotated a further 45°, to result in the head/neck resting horizontal/below horizontal.

prevents bacterial colonization of the trachea, bronchi, and lungs when ETT is elevated 30° above horizontal. A secondary end point was clinical changes in sheep during 72 hrs of mechanical ventilation (MV); we monitored/measured respiratory variables (peak, plateau, and mean airway pressures, Pao_2, $Paco_2$, pH), hemodynamic variables (systolic and diastolic blood pressure, central venous pressure, occlusion pressure, cardiac output); white blood count, body temperature, chest radiograph, blood cultures, and gross findings at autopsy (lung/body weight ratio, purulent mucus in the bronchi, parenchymal consolidation, microabscess/abscess formation). Another secondary end point was safety of this ETT; this included monitoring of the aspiration system throughout the study, analysis of the secretions of the aspiration catheter (bacterial contamination, amount, color, protein concentration), and gross evaluation and histology of the trachea at the level of the aspiration port following 72 hrs of CASS.

Study Design

This study was conducted and approved at the National Institutes of Health animal research laboratory (19). Twenty-two young female Dorset sheep were randomized into three groups.

C Group. In the C group (control group), eight sheep were intubated (standard ETT, Mallinckrodt Hi-Lo) with a standard 8-mm ETT, 72 hrs MV, prone, with head and ETT elevated 30 above horizontal (Fig. 1*a*).

CASS HU Group. In the CASS HU group (CASS, head up), seven sheep were intubated with an 8-mm Hi-Lo Evac, Mallinckrodt endotracheal tube (figure available at http://www.nellcor.com/prod/PRODUCT.ASPX?S1 = AIR&S2 = ETT&id = 162&V = O) and underwent 72 hrs of MV, prone and with the head and ETT elevated 30° above horizontal (Fig. 1*b*). CASS suction was set to ≤20 mm Hg using a continuous low-power suction device (Vacuum pediatric regulator, Ohio Medical Products, a division of AIRCO, Madison, WI).

CASS HD Group. In the CASS HD group (CASS, head down), seven sheep were intubated with the same tube as the CASS HU group and had 72 hrs of MV. CASS was kept at ≤20 mm Hg. Sheep were prone with the ETT and the trachea horizontal or just below horizontal (Fig. 1*b*).

Preparation and Measurements

Anesthesia, MV, humidification, surgical preparation, feeding of the distal gut without use of prokinetics, follow-up, and measurements were obtained as previously described (17, 20). No systemic or topical antibiotics were used at any time. After 72 hrs of MV, sheep were killed with an overdose of sodium pentobarbital.

CASS

The Hi-Lo Evac Mallinckrodt ETT incorporates a separate dorsal lumen to allow for aspiration of subglottic secretions through a large elliptical dorsal opening, proximal to the inflatable cuff. Correct position of the ETT was confirmed radiographically. Subglottic suctioning was continuous, not exceeding 20 mm Hg (21). Every 6 hrs, patency of the drainage was checked and all subglottic secretions in the mucus collector were emptied and recorded. Intracuff pressure was monitored and maintained at 25 cm H_2O.

Tracheal Tube Orientation

When the ETT is placed in the human trachea, irrespective of the supine or semirecumbent body position, there is always a difference of about 10–20 cm between the connector piece (at the top) and the tip of the ETT (at the bottom, in the trachea). To duplicate this orientation in sheep, we lift the neck about 30° above horizontal to have a difference in level of about 15 cm (Fig. 1*a*). In the CASS HD group (Fig. 1*b*), we kept ETT horizontal to possibly simulate a patient in the prone or lateral position (Fig. 1*c*).

Autopsy

The thorax was opened; the lungs were excised, biopsied for bacteriology, and weighed; and gross lung abnormalities were recorded. The trachea was excised, fixed in buffered 10% formalin, and sent to the pathologist for macroscopic and microscopic analysis.

Microbiological Sampling and Microbiological Findings

At the beginning of the studies, samples for colony-forming units/g (17, 20) were collected from the oral cavity (one sample) and subglottic area (one sample). At autopsy, samples were taken from the trachea (one sample), each lobe of the lung (five samples), bronchi (five samples), oral cavity (one sample), subglottic area (one sample), biofilm inside the ETT (one sample), and stomach (one sample). Bacteria were classified as pathogens (opportunistic) or nonpathogens (commensal) by veterinarians of the Laboratory of Animal Medicine and Surgery, National Institutes of Health (22–24).

Statistical Analysis

For continuous variables, we first tested the hypothesis of equality of the three study groups using the Kruskal-Wallis test (25). If the overall two-sided p value was $<.05$, we performed all pairwise comparisons using the Wilcoxon-Mann-Whitney rank test (26) to determine which groups were statistically different. No pairwise comparisons were performed otherwise. A p value of .05 was considered statistically significant. This method protects the overall type I error (false positive) from the multiple pairwise comparisons. For categorical variables, we followed an analogous approach, using the Fisher-Freeman-Halton exact test, which is a generalization of the original Fisher's exact test for cases dealing with tables larger than 2×2 for testing the equality of the three study groups (27). If the two-sided p value of this test was $<.05$, then we performed all the pairwise comparisons. We used the Wilcoxon signed-rank test to assess whether the PaO_2/FIO_2 ratio and bacterial colonization changed from the start of MV to the end (before autopsy) of the study. All tests were two-sided. Results are reported as median and range.

RESULTS

Study Population

We studied 22 sheep (median, 31; range, 25–37 kg; no difference between groups). Sheep were healthy based on clinical criteria, laboratory data, and chest radiograph 1–3 days before the study. Sheep on antibiotics <2 wks before the study were excluded. Intubation was performed without difficulty.

Clinical and Autopsy Findings

In the C group, three sheep were killed before the end of the study after having met end point criteria (Table 1). At autopsy, five of eight sheep showed purulent mucus in the bronchi or parenchymal consolidation or parenchymal microabscess (median lung/body weight ratio was 1.40%, range, 0.98–1.76%).

In the CASS HU group, two sheep met early end point criteria. Upon autopsy, gross findings were found in five sheep. Two other sheep were clinically healthy (except for elevated white blood cell counts). Overall median lung/body weight was 1.24% (range, 0.97–1.60%). Clinically, the CASS HU group did not appear different than the C group.

In the CASS HD group, all seven sheep were ventilated for 72 hrs. Gross appearance of lungs and lung/body weight ratio were unremarkable.

Bacteriologic Findings

Trachea, Bronchi and Lungs. In the C group, the trachea, bronchi, and lungs of all eight sheep were heavily colonized by bacteria. Only 9.1% of the respiratorytract samples showed no bacterial growth, 29.9% of all sites samples were colonized by at least one opportunistic (pathogenic) bacterium, and the remaining 61.0% were colonized by pathogenic multibacterial species.

In the CASS HU group, a lower degree of bacterial colonization was found compared with the C group. However, those differences reached statistical significance only when we considered the total lower respiratory tract count for all bacteria ($p = .05$). Only 5.2% of all samples showed absence of all bacterial growth, and 20.8% of sites were colonized by a single pathogenic bacterial species; the remaining 74.0% were colonized by pathogenic multibacterial species.

In the CASS HD group, there was statistically greatly lower bacterial colonization, compared with the C group and the CASS HU group, in the trachea ($p < .001$), bronchi ($p \leq .001$), and lungs ($p = .001$). In one sheep, pathogenic and nonpathogenic bacterial growth was found; no bacterial colonization was found in all other samples from the other animals (overall, 89.6% of the samples were not colonized by any bacteria, 5.2% were colonized with a pathogenic bacterium, and 5.2% were colonized by nonpathogenic bacteria).

Statistical analysis comparing bacterial growth of all bacteria (pathogens + nonpathogens) and pathogenic bacteria (opportunistic pathogens) in the three groups is shown in Table 2.

ETT, Oropharyngeal Contents, Subglottic Secretions, and the Rumen

Gram-positive and Gram-negative bacteria normally colonized the saliva, subglottic secretions, and rumen in sheep (no differences between the three groups). Pathogenic bacteria found in the lungs at autopsy were always present in oral secretions and in the subglottic secretions at the beginning of the study and at the end. Same bacteria were also found in the ETTs, which were invariably heavily colonized in all sheep of all groups, with no differences among the groups ($p = .12$).

Continuous Aspiration of Subglottic Secretions (CASS). In one study, at the start of suctioning on CASS, we found some blood mixed with oropharyngeal secretions in the aspiration catheter, which cleared after a few minutes. We believethat this blood-tinged material was likely the result of trauma that occurred during intubation rather than from CASS. In the remaining 13 sheep, we observed no blood in the Evac line for the duration of the studies. Median subglottic secretion amounted to 8 mL/hr (range, 2–19 mL/hr). Drainage of secretions was continuous in all CASS studies.

Tracheal Gross Findings

There were no gross tracheal lesions in the control group intubated with standard Hi-Lo ETT (C group) (Table 3).

CASS HU Group. The trachea showed necrosis and/or hemorrhage (Fig. 2*a*) and erythema (Fig. 2*b*) in all seven sheep, in an area immediately adjacent to the dorsal opening of the Evac lumen. Mucosal erosion with submucosal hemorrhage of 8 mm and 6 mm in diameter was found in two sheep; smaller hemorrhages (2–4 mm) were observed in two other sheep. Necrosis of 2–4 mm with exposed cartilage was seen in two studies.

CASS HD Group. Similar to the CASS HD group, all seven sheep showed various levels of tracheal injury adjacent to the Evac suctioning port. In one sheep, cartilage was exposed over a distance of 2 mm in close proximity to the suction port; six sheep showed hemorrhage of the submucosa (2–5mm).

Tracheal Microscopic Findings

In the C group, tracheal epithelium and submucosa at the level of the ETT cuff appeared intact.

In the CASS HU group, the trachea at the level of the suction port showed epithelial erosion in all sheep. Moderate and severe infiltration of neutrophils (Fig. 3*b*), bacteria (Fig. 3*c*), edema, and hemorrhage (Fig. 3*a*) was found in six of seven sheep. In one of the latter the cartilage was exposed. The Elastic Van Gieson preparation revealed universally severe injury with interrupted elastic fibers (Fig. 3*d*).

In the CASS HD group, histologic findings of injury were also observed in this group in all sheep at the level of the suction port, comparable to what seen in the CASS HU group.

DISCUSSION

Based on extensive literature review and personal communication (Mallinckrodt, St Louis, MO), this is the first animal study to evaluate possible benefits and safety of continuous aspiration of subglottic secretions (suction not to ex-

Table 1. Clinical findings during the 72 hrs of mechanical ventilation

	Study Groups		
	C Group (n = 8)	CASS HU Group (n = 7)	CASS HD Group (n = 7)
Hemodynamic instability	4	4	0
Chest radiograph opacifications	5	5	1
Fever	4	4	1
Abnormal WBC counts	7	7	0
PaO_2/FIO_2, median (min–max)			
After intubation	483 (448–529)	480 (457–510)	486 (414–539)
Before autopsy	367 (69–512)	404 (68–486)	453 (346–491)
p value	.04	.02	.02
Gross findings at autopsy[a]	5	5	0
Early criteria for autopsy[b]	3	2	0
Lung/body weight, median (min–max)[c]	1.40 (0.98–1.76)	1.24 (0.97–1.60)	0.92 (0.81–1.10)

C, control; CASS, continuous aspiration of subglottic secretions; HU, head up; HD, head down; WBC, white blood cell count.

[a]Gross findings at autopsy: purulent mucus in the bronchi, parenchymal consolidation or abscess or microabscess formation; [b]early criteria for autopsy: PaO_2/FIO_2 <70 mm Hg, with hemodynamic instability of septic origin requiring infusion of inotropes, confirmed by positive blood culture; [c]normal lung/body weight: median, 1.02; range, 0.88–1.12%.

Table 2. Bacterial colonization of lower respiratory tract (colony-forming units/g of tissue or secretion)

	Study Groups					
	C Group (n = 8)		CASS HU Group (n = 7)		CASS HD Group (n = 7)	
	Median	Range	Median	Range	Median	Range
All Bacteria[a]						
Trachea	3.0×10^9	1.0×10^6–3.2×10^{10}	9.4×10^8	2.8×10^7–7.8×10^9	0	0–0
Bronchi	2.7×10^9	5.6×10^5–6.2×10^{10}	2.4×10^8	2.4×10^6–5.1×10^9	0	0–1.2×10^5
Lung	1.6×10^9	6.5×10^4–4.0×10^{10}	6.1×10^7	7.0×10^6–1.3×10^9	0	0–1.0×10^5
Trachea + bronchi + lungs	1.3×10^{10}	1.6×10^8–1.2×10^{11}	4.6×10^9	1.5×10^8–7.9×10^9	0	0–2.2×10^5
Opportunistic pathogens[a]						
Trachea	3.0×10^9	1.0×10^6–3.2×10^{10}	9.4×10^8	2.4×10^7–7.8×10^9	0	0–0
Bronchi	2.7×10^9	5.6×10^5–3.4×10^{10}	5.3×10^7	2.4×10^6–1.0×10^9	0	0–1.5×10^4
Lung	1.6×10^9	5.3×10^4–4.0×10^{10}	6.1×10^7	7.0×10^6–1.3×10^9	0	0–6.2×10^4
Trachea + bronchi + lungs	1.3×10^{10}	1.6×10^8–8.4×10^{10}	2.4×10^9	1.4×10^8–7.8×10^9	0	0–7.7×10^4

C, control; CASS, continuous aspiration of subglottic secretions; HU, head up; HD, head down.

[a]Pathogenic bacteria: C group: *Moraxella* species (found in eight sheep), *Pasteurella* species (seven sheep), *Pseudomonas aeruginosa* (four sheep), and *Escherichia coli* (two sheep). CASS HU group: *Moraxella* species (six sheep), *Pasteurella* species (six sheep), *P. aeruginosa* (two sheep), *Arcanobacterium pyogenes* (one sheep). CASS HD group: *Pasteurella* species (one sheep). Nonpathogenic bacteria: C group: *Oligella ureolytica* (one sheep). CASS HU group: *Alpha hemolytic streptococcus* species (five sheep). CASS HD group: *Bacillus* species (one sheep). At autopsy, 11 samples were taken from the lower respiratory tract: one from the trachea 2 cm above the carina, five from the five main corresponding bronchi, and five from the five lobes of the lungs. All bacteria refer to the total count of bacteria found in the tissue sample: sum of the nonpathogenic (commensal) bacteria and pathogenic (opportunistic) bacteria.

ceed 20 mm Hg) using the Mallinckrodt tracheal tube Hi-Lo Evac (21).

This study in sheep showed that CASS did not prevent lung bacterial colonization with the head and the ETT elevated 30° above horizontal (CASS HU group; Fig. 1*a*) following 72 hrs of MV. Rello et al. (28) pointed out that subglottic drainage using CASS for the first 48 hrs following intubation did not lower early-onset pneumonia. Yet, previous randomized clinical studies assessed the role of drainage of subglottic secretions in the prevention of VAP. In the most recent study, Smulders et al. (7) demonstrated a decrease from 16% to 4% in the incidence of VAP when intermittent subglottic secretion drainage was used in patients expected to receive mechanical ventilation >3 days. Interestingly, in their standard protocol for infection prevention, they did not use the semirecumbent position, because patient-body position was changed from the left to the right decubitus position every 4 hrs. Unfortunately, the head, trachea, and ETT orientations were not described in the groups.

In our current study, with the same Evac ETT and trachea kept horizontal (Fig. 1*b*; CASS HD group), the trachea, bronchi, and lungs were not colonized, except in one sheep with a low count. We obtained similar results in a previous study (using standard ETTs) without use of the drainage of the subglottic secretions, or suctioning of the ETT, when ETT was kept horizontal and sheep were periodically rotated from right to left lateral body position (17). Despite the use of CASS, raising the ETT 30° above horizontal, as now practiced with the patient

Table 1. Continued

Test for Overall Difference Between Groups p Value	Test for Difference Between Groups: C vs. CASS HU Group p Value	C vs. CASS HD Group p Value	CASS HU vs. CASS HD Group p Value
06	—	—	—
.13	—	—	—
.30	—	—	—
<.001	1.00	.004	.01
.89	—	—	—
.21	—	—	—
.02	1.00	.08	.06
.30	—	—	—
.006	.20	.005	.01

We conclude from our 72-hr animal study that continuous aspiration of subglottic secretions in the manner as now practiced, and as recommended by the manufacturer, did not prevent but only marginally lowered bacterial colonization of the lungs.

Table 2. Continued

Test for Overall Difference Between Groups p Value	Test for Difference Between Groups: C vs. CASS HU p Value	C vs. CASS HD p Value	CASS HU vs. CASS HD p Value
<.001	.42	<.001	<.001
<.001	.25	<.001	.001
.001	.20	.001	.001
<.001	.05	<.001	.001
<.001	.42	<.001	<.001
<.001	.08	<.001	.001
.001	.20	.001	.001
<.001	.04	<.001	.001

resting in the supine or semirecumbent position, may be hazardous, as it allows entry of bacteria-laden mucus into the lower respiratory tract either from the subglottic area or from the bacterial biofilm of the ETT (Fig. 1*a*). While the subject is in the horizontal or below-horizontal orientations of the ETT, the gravitational forces favor continuous removal of secretions/mucus that otherwise could have leaked from the ETT into the trachea and lungs (Fig. 1*b*). In the human, the ETT could be oriented horizontally with the patient prone, but this position may be not tolerated for extended period of time because of the possible pressure necrosis on the forehead, nose, and chin. However, such orientation of the ETT, and of the trachea, could be achieved with the patient in a 45° semilateral position, with the head turned a further 45°. A special bed could rotate the patient periodically from the right lateral position to the left lateral position, and then periodically reverse, as in Figure 1*c*.

Our findings showing severe macroscopic and microscopic injury to the tracheal mucosa/submucosa in all histologic samples at the level of the Evac opening in the CASS group sheep ($p \leq 20$ mm Hg), clearly implicate CASS as the offending agent. None of the seven sheep in the control group using standard ETTs exhibited any tracheal lesions. Recently, Girou et al. (16) showed that 40% of patients, who were intubated with a Hi-Lo Evac ETT (aspiration drainage −30 mm Hg), developed laryngeal edema immediately after extubation, raising questions about safety. A recent personal communication from Mallinckrodt informed us that blood-tinged mucus was found at the time the Mallinckrodt tube Hi-Lo Evac was first introduced to clinical use in Europe. Subsequently, the recommendation was changed for CASS suction not to exceed 20 mm Hg (21). At the suggested suction of <20 mm Hg, we also did not find blood in the aspiration catheter or in the collected secretions, except in one case during the first minutes of the study. Nevertheless, necropsy and histologic findings revealed severe epithelial-mucosal-submucosal tracheal injury adjacent to the CASS suction port after 72 hrs of MV.

However, to interpret our results we have to consider the following. First, results of studies in an animal model are always difficult to extrapolate to clinical practice due to the anatomical and functional differences to the human. Sheep are herbivorous ruminants; normally, the rumen is heavily contaminated by pathogenic and nonpathogenic bacteria (22). Consequently, bacterial colonization of the lower respiratory tract and onset of pneumonia may be overestimated, compared

Table 3. Tracheal findings at the level of the Evac lumen of the Hi-Lo Evac, Mallinckrodt endotracheal tube

	Study Groups				Test for Difference Between Groups		
	C Group (n = 8)	CASS HU Group (n = 7)	CASS HD Group (n = 7)	Test for Overall Difference Between Groups *p* Value	C vs. CASS HU Group*qa* *p* Value	C vs. CASS HD Group *p* Value	CASS HU vs. CASS HD Group *p* Value
Gross findings							
Mucosal erythema or edema	0	7	7	<.001	<.001	<.001	1.00
Submucosal hemorrhage or hematoma	0	4	6	.002	.08	.004	1.00
Mucosal necrosis or exposed cartilage	0	2	1	.27	—	—	—
Microscopic findings							
Epithelial changes, mild	0	3	2	.14	—	—	—
Epithelial changes, severe	0	4	5	.007	.08	.02	1.00
Epithelial changes, any	0	7	7	<.001	<.001	<.001	1.00
Submucosal changes, mild	0	1	1	.52	—	—	—
Submucosal changes, moderate	0	2	4	.04	.60	.08	1.00
Submucosal changes, severe	0	4	2	.04	.08	.60	1.00
Submucosal changes, any	0	7	7	<.001	<.001	<.001	1.00
Exposed cartilage	0	1	1	.52	—	—	—

C, control; CASS, continuous aspiration of subglottic secretions; HU, head up; HD, head down.

Epithelial changes, mild: loss of cilia, erosion or proliferation of the epithelium. Epithelial changes, severe: denudation of the mucosa. Submucosal changes, mild: disruption of the elastic fibers, engorgement of the vessels. Submucosa changes, moderate: hemorrhage and leukocyte and granulocyte infiltration. Submucosa changes, severe: necrosis.

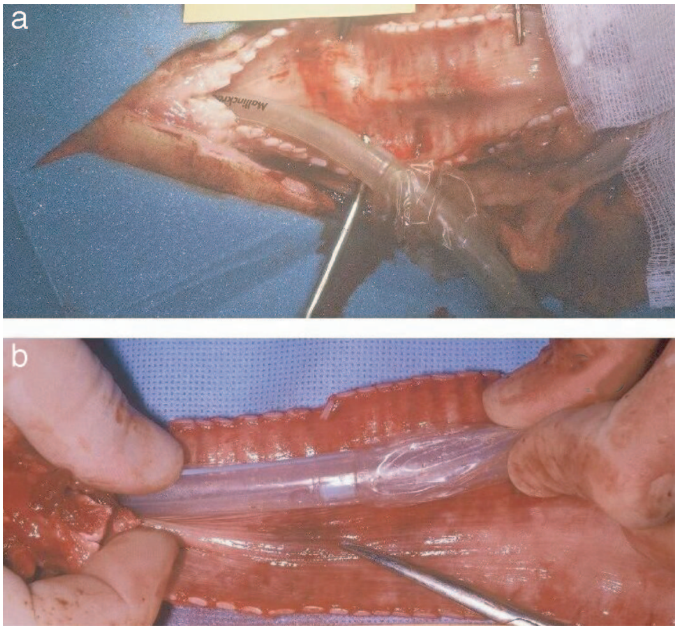

Figure 2. At necropsy, injury of the trachea was observed at the level of the endotracheal tube dorsal opening above the cuff in sheep with continuous aspiration of subglottic secretions ($p \leq 20$ mm Hg). *a*, ulceration, necrosis, and hemorrhage of the tracheal mucosa. *b*, submucosal hemorrhage and erythema of the tracheal mucosa.

with the human. At the same time this animal model has many advantages:

- Studies were conducted in absence of antibiotics; low-volume, low-pressure MV was used; humidified and heated disposable respiratory circuits were adopted; and feeding was delivered throughout the study into the rumen.
- Bacterial colonization of the lungs was not confounded by previous episodes of pneumonia or by coexisting noninfectious lesions.
- Multiple samples can be obtained by direct lung biopsy at autopsy.
- The trachea was assessed immediately after death with histologic studies.

Second, histologic studies are lacking. Our 72-hr study focus was designed to determine whether CASS prevents bacterial colonization of the lower respiratory tract, rather than decreasing the incidence of VAP, during a prolonged course of MV. However, CASS through the Hi-Lo Evac ETT should prevent the leakage of secretions around the ETT cuff and entry of bacteria in the lungs. A new medical device for the prevention of VAP should be designed/built to eliminate the offensive agent safely. In the case of bacterial pneumonia, bacteria are the offensive agent.

Third, the difficulty in the clinical diagnosis of VAP and the absence of gold standard for VAP diagnosis (1, 2) led us to explore bacterial colonization of the lungs through direct biopsy of the trachea, the five main bronchi, and the parenchyma of the five lobes of the lungs at elective autopsy; we chose this rather than rely on bronchoalveolar lavage, bronchoscopy-guided protected specimen brush, or tracheal aspirate, now widely used in the clinical setting.

Fourth, in every study in which CASS was used, we found tracheal injury within 72 hrs, irrespective of the position of the neck (ETT and trachea horizontal, or ETT and trachea elevated 30°). Although sheep were prone and their necks are anatomically different than the human neck, our results now cast doubts on the clinical safety of CASS.

Fifth, we did not investigate the role of intermittent drainage of subglottic secre-

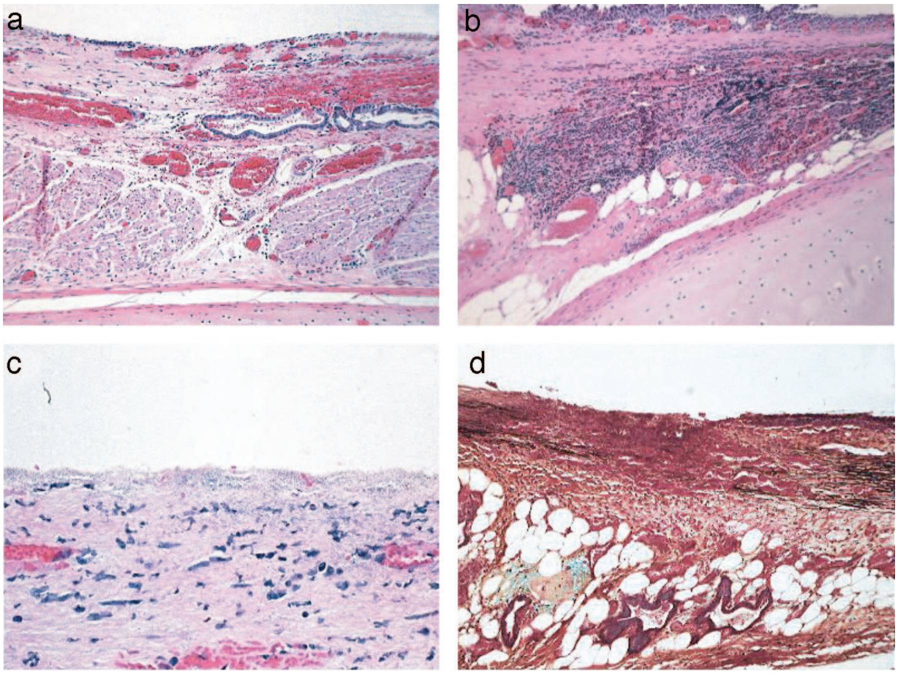

Figure 3. Tracheal histology at the site of the dorsal opening of the evacuation lumen with continuous aspiration of subglottic secretions, aspiration pressure ≤ 20 mm Hg. *a*, note denudation of the mucosa, engorgement of vessels, and hemorrhage. *b*, epithelial proliferation, engorgement of vessels, and intense infiltration of inflammatory cells. *c*, total denudation of the epithelium, with bacterial infiltration of the submucosa. *d*, Elastic Van Gieson staining. Epithelium is denuded, elastic fibers are damaged and interrupted (in black); note hemorrhage, inflammation, and necrosis.

tions. It may be possible that intermittent aspiration of the subglottic secretions is not as injurious as continuous aspiration of the subglottic secretions at 20 mm Hg, as suggested by Smulders (7).

Sixth, we did not perform a double-blinded study.

Seventh, this randomized laboratory study was composed of three study groups. The head-down group intubated with a standard ETT was not included in this study, as it had been already previously reported (17, 18). However, the specific intent of this laboratory study was to explore the possible benefit of the CASS in preventing bacterial colonization of the lower respiratory tract with the ETT raised 30° above horizontal (as in patients in the semirecumbent/supine position). This study confirms our previous studies that showed the horizontal orientation of standard ETT, alone and without use of CASS, to prevent bacterial colonization of the lower respiratory tract following 72 hrs of mechanical ventilation, whereas standard ETT elevated 30° from horizontal had overwhelming bacterial colonization of the tracheobronchial structure and lungs, a high incidence of nosocomial pneumonia, and death—as in this study in the CASS HU group.

CONCLUSIONS

We conclude from our 72-hr animal study that continuous aspiration of subglottic secretions in the manner as now practiced, and as recommended by the manufacturer, did not prevent but only marginally lowered bacterial colonization of the lungs. Furthermore, CASS is the direct cause of major tracheal injury at the site of applied subatmospheric pressure in our animal model. However, the orientation of the ETT to horizontal/below-horizontal alone prevented entry of bacteria into the lungs, solely through the action of gravitational forces. Further laboratory studies need to confirm our results and explore a safe range of continuous aspiration with no mucosal injury. We hope this study may stimulate investigators to explore the role of horizontal orientation of the ETT in the clinical setting.

ACKNOWLEDGMENTS

We thank Antoni Torres, MD, Joel Moss, MD, and Martha Vaughan, MD, for helpful comments and for reviewing this manuscript. We also to thank Mario Stylianou, PhD, mathematical statistician, Office of Biostatistics Research, NIH, for the statistical analysis. In addition we acknowledge the staff of the Laboratory for Animal Medicine and Surgery, NHLBI-NIH, and of the Veterinary Resources Program, ORS-NIH, for the assistance throughout the animal studies and the bacteriologic analysis: Dr. Robert F. Hoyt, Randall Clevenger, Kelly Cole, Dave Caden, Phyllis Levin, Arthur Zetts, Timothy Hunt, Kenneth Jeffries, Karen Keeran, Gayle Zywicke, Shannon Whitmire, Paula Buford, and Fran Benedetti.

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